

MYCORRHIZAE OF TREES WITH SPECIAL EMPHASIS ON PHYSIOLOGY OF ECTOTROPHIC TYPES

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Most absorbing roots of forest trees in their native habitats are invaded by specific nonpathogenic fungi; together the roots and fungi comprise very orderly morphological units, the mycorrhizae. Physiologically, mycorrhizae represent cases of symbiosis, or reciprocal parasitism, but not of pathogenesis. To give an insight into our present knowledge of the physiological interactions of trees and mycorrhizal fungi, I shall first discuss briefly some of the morphological characteristics of mycorrhizae.

CLASSES OF MYCORRHIZAE

On the basis of the interrelation between the fungus hyphae and the root cells, mycorrhizae are classed in two main groups, ectotrophic and endotrophic. The kind is usually specific for a tree genus. Typical ectotrophic mycorrhizae are caused by invasion of actively growing absorbing roots usually by hymenomycetous, but sometimes by ascomycetous, fungi. These fungi usually form compact mantles of mycelium on the surface of the roots. Subsequently they dissolve the middle lamellae of the epidermal and outer cortical cells and then the hyphae surround the walls of those cells. The living epidermal and cortical cells are thus isolated by mycelial partitions. The diameters of the invaded roots are increased, but the cell volumes are not changed. In sectioned material the pattern formed by hyphae in the cortex is referred to as the Hartig net. The meristematic tip and the stele of the root are not invaded by hyphae. The infected roots are shorter than those uninfected, sometimes are branched, and do not develop root hairs. Ectotrophic mycorrhizae are commonly found on pine, spruce, oak, elm, beech, hickory, chestnut, birch, and other trees.

Endotrophic mycorrhizae are caused by the invasion of absorbing roots by specific phycomycetous fungi. The hyphae are present on the root surfaces only as individual threads and penetrate directly into the root hairs and other cells of the epidermis. Penetration of cells sometimes extends no farther than the epidermis; frequently, however, the hyphae grow into the cortex cells. Within the cells the hyphae may appear as coils, swellings, or minute branches. As in ectotrophic mycorrhizae, there is no penetration of meristem or stele. The gross morphology of the invaded roots may remain unchanged or the roots may become beaded, the beading probably being caused by periods of arrested growth of the roots followed by periods of active growth as the result of changes in environmental conditions. Endotrophic mycorrhizae are commonly found on yellow poplar, maple, sweet gum, and ericaceous plants.

Besides the two types of mycorrhizae just described, there occasionally appears on tree roots the typical organization of the ectotrophic mycorrhizae plus intracellular penetration by hyphae. These ectendotrophic mycorrhizae are sometimes thought to represent a transitional stage between the ectotrophic and the endotrophic type but may represent either a pathogenic tendency on the part of an ectotrophic fungus under conditions unfavorable to the host or a secondary invasion by fungi of a weakly parasitic nature brought on by alterations in the rhizosphere.

Sometimes invasions by pathogenic fungi may change the gross morphology of absorbing roots so that they appear similar to ectotrophic mycorrhizae. Hyphal penetration, however, is not restricted to specific tissues and ultimately results in

death of the roots. Sometimes referred to as pseudomycorrhizae, these are clearly cases of pathogenesis detrimental to the host.

FORMATION OF MYCORRHIZAE

Investigators of the physiology of mycorrhizal associations have long sought to learn their significance and understand the mechanisms involved. From the results of many isolated experiments in different parts of the world it is now possible to synthesize a reasonable, partial explanation of the mechanisms of the association.

The first requisite for the formation of mycorrhizae is, of course, contact between actively growing roots and compatible fungi. The contact may originate from spores germinating in the vicinity of the roots, by extension through the soil of hyphae from either residual mycelium or established mycorrhizae, or by progression of hyphae through internal root tissues. Thereafter many interactions are initiated between the fungi and the root cells.

Growth of mycorrhizal fungi on the surfaces of the roots is greatly stimulated by exudates from the roots. These exudates contain at least one growth-promoting metabolite, which the discoverer designated as the M-factor. This substance has not been identified, but it is not any of the known B vitamins, the purine and pyrimidine bases or the amino acids in casein-hydrolysate. Dependency on the M-factor varies widely with the various species of mycorrhizal fungi. Use of the metabolite will probably greatly assist in culturing mycorrhizal fungi which have resisted all attempts at isolation and maintenance in pure culture.

Entrance of ectotrophic mycorrhizal fungi into the roots requires secretion of pectolytic enzymes, which dissolve the middle lamellae and thus permit the hyphae to grow through the intercellular regions of the cortex. Endotrophic mycorrhizal fungi secrete cellulolytic rather than pectolytic enzymes. By penetrating the cell walls the hyphae enter the intracellular regions of the cortex cells. Fungi which cause ectendotrophic mycorrhizae secrete both pectolytic and cellulolytic enzymes. Physical or chemical properties of the roots, or perhaps both, restrict the hyphae of all mycorrhizal fungi to the cortex delimited by the endodermis and meristematic cells of the root tip. The mechanism of this resistance to the hyphae is not known.

The growing, absorbing roots of several species of forest trees which have become enveloped by ectotrophic mycorrhizal fungi are subjected to a growth-regulatory substance, which may be indoleacetic acid. This substance causes modification of subsequent root growth. It retards elongation of the roots and frequently initiates dichotomous branching. The end result may be very dense coralloid formations of mycorrhizae. The cortex cells are oriented somewhat differently from those in non-mycorrhizal roots, and presence of intercellular hyphae causes a swollen appearance. There is, however, no increase in the cell volume. It is probable that formation of new absorbing roots is initiated by the growth-regulator. When root systems of responsive species are placed in liquid culture media or extracts thereof, branching occurs even without mycorrhizal formation. The branched absorbing roots and the mycelium radiating from the surfaces of mycorrhizae greatly increase the absorbing potentiality of any particular mycorrhizal root. Trees with endotrophic mycorrhizae apparently do not respond to the secretions of the growth regulator. This leads us to believe that trees having ectotrophic or endotrophic mycorrhizae are different physiologically and that this inherent difference determines the type of mycorrhizal association into which the species will enter.

EFFECTS OF SOIL ENVIRONMENT

Soil Acidity

Mycorrhizae of trees develop most extensively in acid soils, probably because all mycorrhizal fungi of trees studied for pH requirements are acidophilic. However, different species have different optima for maximum growth.

Organic Growth Factors

Several other soil factors besides acidity strongly affect development of tree mycorrhizae. There is evidence that certain vitamins and amino acids, for which mycorrhizal Hymenomycetes and Gasteromycetes are heterotrophic, are available in the soil in sufficient amounts for maximum growth of the fungi. These may originate as products of the metabolism of other microorganisms during decomposition of organic matter. There is also evidence that substances inhibitory to mycorrhizal fungi are produced by microorganisms in the soil and that other inhibitors are present in leaf litter of aspen, beech, maple, oak, and pine.

Temperature, Aeration, and Moisture

The greatest frequency of mycorrhizal formation is during the spring. Formation then decreases in early summer, but there is a second peak during early autumn. Abundance of new mycorrhizae may be correlated with higher soil moisture levels during spring and autumn as compared with those in summer, when rainfall is less and transpiration rates are high. Low temperatures during mid-winter limit the development of mycorrhizae by suppressing root and fungus growth. In late winter and very early spring while the shoots of trees are apparently dormant, roots may grow extensively and be invaded by mycorrhizal fungi. Thus, by the time the buds begin to swell, the roots are in a very active state.

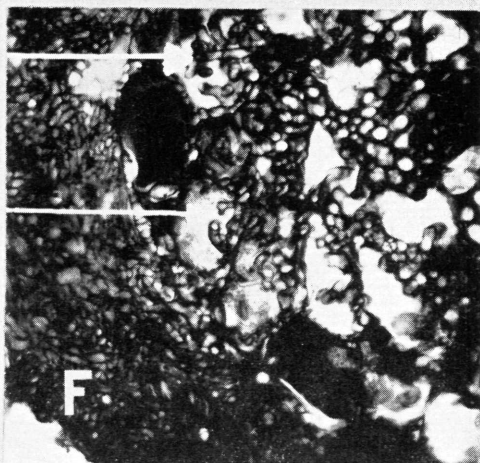
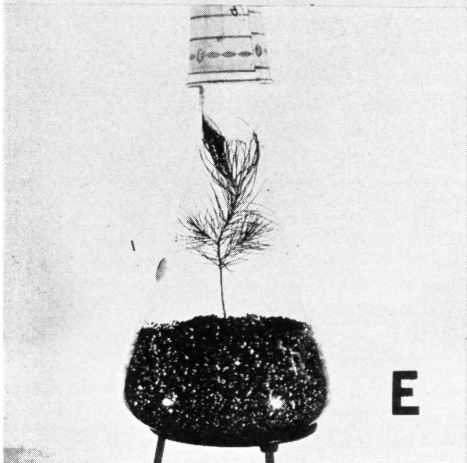
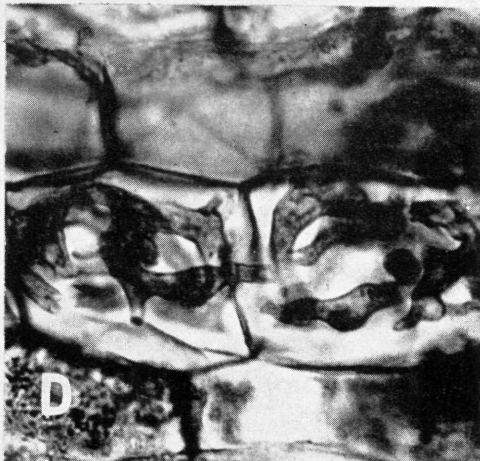
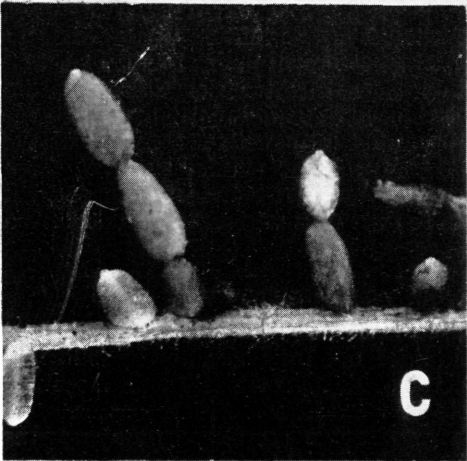
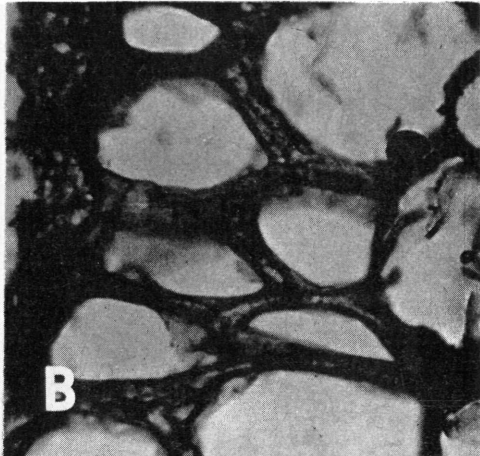
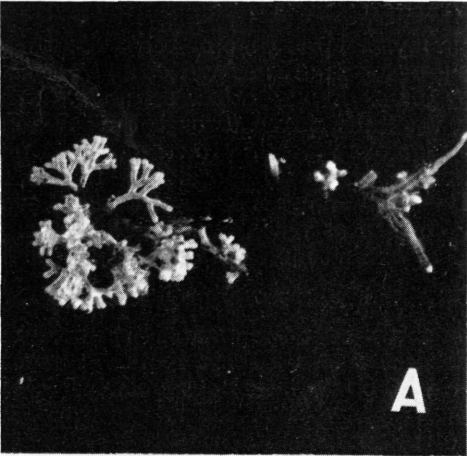
For good development of mycorrhizae soils should be well aerated. Heavy clays reduce root and fungus growth by decreasing aeration while light-textured soils may do the opposite. The balance between soil moisture, aeration, and temperature can determine the complement of microorganisms and in all probability the species of fungi which will enter into the state of mycotrophy under a given set of conditions. More attention should be given to those factors as they influence formation and physiological activity of mycorrhizae.

Nutrient Level

The available inorganic ions in the soil have a profound effect on mycorrhizal formation if all other factors are optimal. The results of several investigators consistently show that formation of ectotrophic mycorrhizae varies inversely with soil fertility. This appears to be especially true when related to the available phosphorus, nitrogen, and potassium in the soil. In soils which accumulate large amounts of humus and consequently have low amounts of soluble nutrients, mycorrhizal formation is most profuse and the mycorrhizae are most effective in stimulating the growth of trees. When decomposition of humus is rapid and the available nutrient level high, mycorrhizal formation is relatively less. Addition of inorganic fertilizers to soils may completely suppress formation of ectotrophic mycorrhizae. Little is known as to the effects of available nutrients on formation of the endotrophic mycorrhizae.

EXPLANATION OF FIGURES IN PLATE

- A. Coralloid ectotrophic mycorrhizae of *Pinus virginiana*.
- B. Cross-section of an ectotrophic mycorrhiza of *P. virginiana* illustrating intercellular Hartig net.
- C. Beaded form of endotrophic mycorrhizae of *Acer rubrum*.
- D. Longitudinal section of an endotrophic mycorrhiza of *A. rubrum* illustrating intracellular hyphae.
- E. Seedling of *Pinus virginiana* grown aseptically on a vermiculite substrate.
- F. Cross-section of an ectendotrophic mycorrhiza of *P. strobus* illustrating Hartig net and intracellular hyphae.



MINERAL NUTRITION

The earliest indications that ectotrophic mycorrhizae were of benefit to trees appeared in experiments wherein seedlings growing in soils devoid of mycorrhizal fungi ceased growth after one or two years. When other seedlings with mycorrhizae were interplanted in the beds, the fungi spread through the soil and invaded the uninfected roots. Thereafter vigorous renewed growth occurred on the retarded seedlings. Similar results were obtained by inoculating the beds with duff containing mycorrhizal fungi. Chemical analyses of plant tissues have shown that plants with mycorrhizae may contain as much as 86 percent more nitrogen, 234 percent more phosphorus, and 75 percent more potassium than those without mycorrhizae. In recent years radioactive isotopes of several essential elements have been used to show their transfer by mycorrhizal fungi from the substrate into roots and their translocation throughout the plant. Mycorrhizae also accumulate greater amounts of phosphorus than do short roots with root hairs. This accumulation is closely related to the respiration of mycorrhizae.

INFLUENCES OF LIGHT

The effects of light on mycorrhizal formation are manifested indirectly as a result of the response of the host to light intensity. Formation of mycorrhizae may be reduced or completely suppressed on plants grown under low light intensities. Girdling of stems, which results in interrupting translocation of carbohydrates to the roots, inhibits mycorrhizal formation. This is correlated with the amount of carbohydrates in the roots and subsequently with the effects of the carbohydrates on the nutrition of the fungi. Logically, species requiring high light intensities should be at a disadvantage as regards mycorrhizal formation if grown in the shade. There is little evidence to date that variation in photoperiod influences formation of mycorrhizae.

STATUS OF CURRENT KNOWLEDGE AS TO RECIPROCAL
EXCHANGES IN MYCORRHIZAE

From experimental evidence thus far obtained one may assume the following regarding the reciprocal effects of roots and ectotrophic mycorrhizal fungi. Accumulation of soluble carbohydrates in the roots of trees in some instances depends largely upon light intensity and duration and favors secretions of a growth-stimulating metabolite into the rhizosphere. Mycorrhizal fungi, usually Hymenomycetes or Gasteromycetes, present in the rhizosphere are stimulated by the metabolite and invest actively growing short roots. Secretions of an auxinlike compound by the fungi cause the absorbing roots to develop into short, sometimes profusely branched structures, especially on pines. Secretions of pectolytic enzymes enable the fungus to enter the intercellular regions of the cortex only. Restriction of hyphae to the cortex and the entrance of specific fungi into the roots are controlled by unknown mechanisms. The hyphae, which frequently radiate some distance into the soil adjacent to the roots, absorb and translocate nutrients from the soil into the root tissues in greater quantities than do roots without mycorrhizae. In exchange, the fungi utilize carbohydrates and probably other growth substances from the root cells. This exchange may be altered by several environmental factors including the available nutrients in the soil and the light intensity.

Fertilizers added to the soil are readily absorbed by the tree. When they are translocated upward near the source of the photosynthate, soluble carbohydrates are assimilated rapidly during formation of new protoplasm and cell walls in the shoot. The amounts of soluble carbohydrates translocated to and accumulated in the roots, and of the M-factor secreted, would be low. Formation of mycorrhizae would not be favored under these conditions. Reduction in light intensity would

not favor shoot growth but would result in a similar low amount of soluble carbohydrates in the roots and would suppress mycorrhizal formation.

Since little more than morphological studies have been made on the endotrophic mycorrhizae of trees, the physiological aspects of the relationship are not well established. It has been observed, however, that hyphae which invade the root cells follow the advancing meristematic root tip and grow from cell to cell. The older hyphae are then digested by the root cells and probably the contents of the hyphae are assimilated by the host. Cytochemical studies may be used in the future to assist in determining the physiological interactions.

PRACTICAL APPLICATIONS

Seed of some species of trees introduced into certain parts of the world must be complemented with suitable mycorrhizal fungi which are not indigenous there. This may be accomplished by starting the seedlings in beds into which mycorrhizal fungi have been introduced. Interplanting with seedlings having mycorrhizae is effective also. In Sweden it is recommended that fertilizing of the seedlings should be done sparingly if the trees are to be transplanted where they will not be fertilized. In this country it would also seem advisable to encourage a good mycorrhizal system before transplanting; high rates of application of fertilizer produce larger stocks but few mycorrhizae and later, when the plants are becoming established, difficulties may result.

When seed beds are being prepared, it is now common practice to control soil pathogens by application of chemicals to the soil. Some chemicals may cause stunting of the seedlings by direct injury to the plants. Others, when used in high concentrations, can eliminate both soil pathogens and mycorrhizal fungi. The reduction of competition for soil nutrients results in larger seedlings. It would be advisable under such conditions to inoculate the beds with mycorrhizal fungi to permit formation of mycorrhizae prior to transplanting. Other chemicals may reduce the population of organisms and prevent serious losses of seedlings but do not kill enough of the mycorrhizal fungi to prevent their redistribution in the seed bed and on the roots. The soil treatments most widely used at present seem to do the latter.

TRENDS OF INVESTIGATIONS

Physiological studies of mycorrhizae, though complicated and requiring the development of new techniques, should be performed both under controlled laboratory conditions and in the field to clarify further the functions of mycorrhizae. The place of endotrophic mycorrhizae in successful nutrition of some trees and the effects of pH, soil nutrients, light and other factors on their formation should be determined. Our lack of knowledge about endotrophic forms leaves much to be investigated.

Fungi forming ectotrophic mycorrhizae are known to vary in their physiological requirements and therefore may be expected to vary in their efficiency as partners in the mycorrhizal associations. Research designed to select the most efficient fungus associates of the various tree species for use in various soil types is indicated.

In preliminary experiments we found indications that the auxin effects on root systems vary with species of trees depending upon whether the mycorrhizae are ectotrophic or endotrophic. There is indicated the need for basic studies on the differences between roots of trees in their responses not only to ectotrophic and endotrophic mycorrhizal fungi but to other root invaders.

Synthetic growth-regulators can probably be used to initiate new roots on transplants if a practical method is devised for treating them. Such seedlings inoculated with mycorrhizal fungi should be in a better condition for survival than uninoculated ones. In preliminary studies, new roots were initiated on pine

seedlings and converted to mycorrhizae when indoleacetic acid and pure cultures of mycorrhizal fungi were used.

It would be of interest to know whether the fungi secreted metabolites of an antibiotic nature which might retard growth of other soil organisms as well as affect the metabolism of the host.

Controlling diseases by use of biocides in soil treatments and of systemic protectants is a goal of plant pathologists. We know that the soil treatments do affect mycorrhizal fungi. We now wonder whether it would be possible to reduce greatly the microflora and fauna in the soil, permit the tree seedlings to become established under conditions of reduced competition for nutrients, and then inoculate the seed beds with pure cultures of the most efficient mycorrhizal fungi. It will of course be necessary first to screen the fungi for relative efficiencies. In any case we hope that the mycorrhizal association will not be overlooked so that latent damage by biocides to the absorptive systems will not exceed the initial benefits from control of disease and reduction of competition.

In conclusion, one might state that a given tree species will grow if climatic and soil conditions permit. However, growth will be best when biotic conditions, and especially the natural development of the mycorrhizal association, are best. Better growth can be obtained if biotic relations are worked out through studies of mycorrhizae and the principles are applied to practical problems.

DISCUSSION

WM. H. DAVIS MCGREGOR (*U. S. Forest Service and Duke University*): Do you have any evidence that the micorrhizal fungi produce indoleacetic acid?

EDWARD HACSKAYLO: The growth-regulatory substance which causes dichotomous branching of ectotrophic mycorrhizae has not been specifically identified as indoleacetic acid. We do know, however, that at least one species of *Boletus* does produce indoleacetic acid and that other fungi may secrete organic acids including indoleacetic acid. At present we are assuming that this is the substance causing the morphological changes.

DENNIS RICHARDSON (*University of Aberdeen, Scotland*): I do not believe we have any evidence of a fundamental difference in root physiology between trees which form ectotrophic mycorrhizae and those forming endotrophic association. Such a difference has been adduced from the fact that the formation of uninfected roots closely resembling mycorrhizae can be produced as a result of adding auxin to the growth medium in the case of ectotrophic forms, but not in that of endotrophs. This is true, but there is evidence in the case of *Acer* that such roots can be produced by the addition of auxin to the terminal shoot of a decapitated and defoliated seedling.

It was also suggested that there is a difference in root physiology because, *e.g.*, ectrophic mycorrhizal fungi do not infect roots of endotrophic trees and if there were no difference dual infection should be possible. This, I think, is a dubious conclusion; could it not be that the difference lies in the fungi (which are known to differ in *e.g.* the enzymes they secrete) rather than in the roots? Or be due to differences in environmental requirements between endotrophic type roots and ectotrophic type fungi?

EDWARD HACSKAYLO: Species of trees which have either ectotrophic or endotrophic mycorrhizae are frequently found growing adjacent to each other. The fungi involved in each of those associations are present in the soil and yet maintain specificity in the root penetrations. Why then do not the fungi which cause ectotrophic mycorrhizae and secrete pectolytic enzymes penetrate roots of trees which are invaded by only the cellulolytic enzyme-producing fungi? I am more inclined to believe that it is a host-resistance to the fungi which points toward a physiological diversity in the tree species. If differences in environmental require-

ments are involved this would again strongly suggest that the differing responses of the hosts to a given environment has a physiological basis.

DENNIS RICHARDSON: Would you agree with the contention first stressed by Harley that mycorrhizae cannot be regarded as unique examples of plant-micro-organism inter-relationships? That, in fact, a mycorrhiza is just one of many such associations between a plant root and a component of the rhizosphere population?

EDWARD HACSKAYLO: Certainly mycorrhizae are very widespread and I would be the first to acknowledge that they are only one component of a complex population of micro organisms in the rhizosphere which must be exerting influences on each other and on the higher plants.

A. G. SNOW (*U. S. Forest Service*): You mention complete separation of cortex cells by Hartig net. Are there any cyto-protoplasmic strand connections also between these cortex cells?

EDWARD HACSKAYLO: We do not know whether there might be connections similar to plasmodesmata between the cortex cells. Possibly stain techniques could be used to establish the answer to that question.
